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(54) Title: LIPOSOME-BASED TOPICAL TRETINOIN FORMULATION

(57) Abstract

A liposome-based tretinoin formulation with good skin penetration of the effective substance is described. This formulation is well suited for the treatment of acne or photoaging.

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LIPOSOME-BASED TOPICAL TRETINOIN FORMULATION

FIELD OF THE INVENTION

The present invention relates to a liposome based topical formulation of tretinoin, particularly a formulation providing good penetration of biological active substances into the skin, suitable for the treatment of acne, psoriasis and photoaging. In particular, the invention relates to a liposome based tretinoin formulation with superior stability prepared by means of an ethanol injection technique.

BACKGROUND OF THE INVENTION

It has been known for some time that retinoic acid and its derivatives are effective therapeutic agents in topical treatment of acne and other skin disorders, because it decreases the cohesiveness of follicular epithelial cells and induces proliferation of the follicular epithelium. More recently, retinoic acid has been used topically for the treatment of photoaging of the skin. Presently, the topical formulations in use are preferably conventional creams, liquids or gel based formulations which are marketed in the United States under the trade names RETIN-A® and RENOVA®. These products contain tretinoin (trans-retinoic acid) in concentrations of 0.025%, 0.05% and 0.1% creams and 0.01% and 0.025% gels.

While these formulations have been proven to be highly successful, certain side effects have been observed including irritation with redness, inflammation and local erythema and peeling. In addition, systemic toxicity may be associated with excess absorption of the active ingredient. Further, in general, these formulations exhibit limited stability with a shelf-life of not more than three years.

Accordingly, attempts have been made to provide an improved formulation of tretinoin which provides effective penetration of the active ingredient into the skin yet minimizes systemic absorption. At the same time, an acceptable formulation should be cosmetically acceptable and exhibit a high degree of patient compliance. Further, the formulation should be stable and provide and extended shelf life.

Liposomes are small vesicles comprising amphipathic lipids arranged in spherical bilayers. Liposomes may contain many concentric lipid bilayers separated by aqueous channels (multilamellar vesicles or MLVs), or alternatively, they may contain a single membrane bilayer (unilamellar vesicles), which may be small unilamellar vesicles (SUVs) or large unilamellar vesicles (LUVs). The lipid bilayer is composed of two lipid monolayers having a hydrophobic "tail" region and a hydrophilic "head" region. In the membrane bilayer, the hydrophobic "tails" of the lipid monolayers orient towards the center of the bilayer, whereas the hydrophilic "heads" orient toward the aqueous phase.

Liposomes may be used to encapsulate a variety of materials by trapping hydrophilic compounds in the aqueous interior or between bilayers, or by trapping hydrophobic compounds within the bilayer. As such, they are particularly useful to deliver biologically active materials by encapsulating compounds which exhibit poor aqueous solubility or which exhibit unacceptable toxicity at therapeutic dosages. Topical liposome formulations have been known for years and topical retinoic acid liposomal preparations have been proposed. For example U.S. Patent 5,034,228 discloses liposomal low dose tretinoin formulations in which the liposomes are prepared by methods involving the use of dichloromethane solvents and spray drying followed by size homogenization with ultrasounds or high pressure homogenization. The use of dichloromethane however is undesirable and the high temperatures and pressures used in the liposome sizing techniques can have an adverse effect on the resulting product.

A specific method for the production of liposomes with only one double layer is disclosed in EP 253 619.

The goal of the present invention therefore was to provide a topical application form suitable for tretinoin, which is cosmetically elegant and minimizes irritating side effects, and which provides good skin penetration abilities yet minimizes skin permeation and systemic absorption. Independently therefrom, the formulation should also provide superior long term stability for an extended shelf life in comparison with known formulations.

SUMMARY OF THE INVENTION

A liposome-based composition for use in the topical treatment of skin disorders comprising:

(a) an effective amount of an active ingredient comprising tretinoin or its pharmaceutically acceptable derivatives;

(b) a lipidic phase comprising:

(I) lecithin or hydrogenated lecithin; and

(ii) cholesterol or a derivative thereof selected from cholesterol esters, polyethylene glycol derivatives of cholesterol (PEG-cholesterols), and organic acid derivatives of cholesterols; and

(c) a lower alcohol (preferably ethanol);

wherein the composition comprises single bilayered liposomes made by preparing an alcoholic solution of the lipidic phase and the active ingredient and injecting the solution under pressure into an aqueous electrolyte solution contained in a high speed homogenizer.

Preferably, the active ingredient is tretinoin (all *trans* retinoic acid) and its derivatives, salts and esters. These compounds, their chemistry, and synthesis, are described in Frickel, F., Chemistry and Physical Properties of Retinoids: THE

RETINOIDS, Sporn, Roberts, Goodman eds., Academic Press, p. 7-145 (1984), hereby incorporated by reference.

In accordance with the present invention, it has been discovered that, quite unexpectedly, the liposomal tretinoin compositions prepared under the mild conditions described herein exhibit improved stability, i.e. the liposomes themselves are stable and at the same time the chemical degradation of the biologically effective substance is minimized. As a further unexpected advantage, the liposomal tretinoin compositions of the instant invention provide a high level of skin penetration to achieve the therapeutic effect while allowing virtually no skin permeation thereby minimizing the risk of systemic side effects.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 - shows the results of in vitro skin permeation studies of various formulations showing the level of tretinoin in the skin 24 hours following application of the formulation, and

FIGURE 2 - shows the results of in vitro skin permeation studies of various formulations showing the level of tretinoin which had permeated through the skin to the receptor 24 hours following application of the formulation.

DETAILED DESCRIPTION

The active ingredients used in the present invention are the tretinoin (all trans retinoic acid) compounds in general, their salts and esters or mixtures thereof. The compositions are useful in treating dermatological disorders including acne, photoaging, wrinkles, hyperkeratosis, psoriasis, to lighten or remove pigmental skin spots, and the like. As used herein, photoaging means damage to essential structural and functional components of the skin resulting from chronic exposure to ultraviolet radiation. Clinical signs of photodamage include wrinkling, mottled hyperpigmentation and roughness accompanied by histologic changes such as epidermal atypia, breakdown of elastin and collagen fibers in the dermis and increased melanocytic activity.

The liposome compositions generally contain from about 10mg to about 1000mg of the retinoic acid compound per 100 grams of composition. Such a formulation, particularly produced according to the process described in EP 0 253 619, which is herein incorporated by reference, shows very good penetration abilities of retinoic acid and its derivatives and related compounds, particularly tretinoin, when applied in topical application.

Lecithin can either be used as natural lecithin in purified form or, preferably, as the more stable hydrogenated lecithin, whereby the use of the latter allows a reduction of the concentration of the stabilizing agents. The lecithin component is generally present in an amount from about 1.0 to 10 grams per 100 grams of composition. Preferably, the hydrogenated lecithin should be of good quality without detectable levels of catalysts which can influence the stability of tretinoin and liposomes in a negative manner.

Cholesterol is employed as the liposome stabilizing agent in amounts ranging from 0.1 to 1.0 grams per 100 grams of composition. In addition to cholesterol,

other cholesterol derivatives may be employed such as cholesterol esters, polyethylene glycol derivatives of cholesterol (PEG-cholesterols), as well as organic acid derivatives of cholesterols, for example cholesterol hemisuccinate.

The alcohol component is a lower alkanol of one to six carbon atoms, such as methanol, ethanol, n-propanol, isopropanol, n-butanol and the like in amounts ranging from 0.5 to about 8.0 grams per 100 grams of composition. Ethanol is preferred.

Dependent on the amount to be applied and/or the place of application, the use of highly fluid products for topical application of retinoic acid is unfavorable. It is therefore advantageous to include a gelling agent in the composition to provide a less fluid product. Various gelling agents may be employed and are within the scope of this invention including cellulose derivatives such as hydroxypropyl methylcellulose. In particular, however, it has been found that a liposomal formulation as described above, but additionally comprising one or more a polyacrylate(s) such as carboxypolymethylene (carbomer) as gelling agent, makes possible a much better skin penetration of the active ingredient than do paraffin ointment bases or liposome-based formulations with other gelling agents such as xanthan gum. By the use of polyacrylate(s) as gelling agent(s), the penetration abilities of the highly fluid liposome-based formulations are at least reached or even enhanced.

In a topical retinoic acid composition, it is important to achieve the proper balance of skin penetration of the active ingredient while minimizing the permeation of the active ingredient through the skin where it can be systematically absorbed. For the topical compositions such as creams, the skin penetration is rather low. On the other hand, for those compositions where skin penetration is high, permeation is also high and therefore the danger of systemic effects is a problem. The instant invention, in contrast, provides good skin penetration to achieve the therapeutic effect while allowing virtually no skin permeation. In this manner, the number of administrations

per day can be minimized due to higher penetration and delivery of the active ingredient to the skin while minimizing side effects due to systemic absorption.

As stated above, the topical tretinoin compositions currently marketed have limited shelf-life of not more than three years. For those topical liposomal tretinoin which have been reported in the literature, stability measurements of only up to six months could be found. (Maingnen F. et al, J. Phar. Clin. 14, No. 2:137-138 1995). In contrast and quite surprisingly, it has been found that the liposomal compositions of the present invention containing carbomer gelling agent exhibit improved stability, i.e. the liposomes themselves are stable and at the same time the decomposition of the biologically effective substance is minimized. A shelf-life of up to five years has been achieved which is very important for industrial application. This improved stability may be attributable to the superior mild manufacturing technology of the present invention and the ingredients and composition of the formulation (both from a qualitative and quantitative point of view when compared with the formulations described in the literature). Particularly, it is theorized that the normally high pressure homogenization or the french press methods used in other prior art methods of manufacturing liposomes result in high temperatures (up to more than 100°C) and pressures (up to 800 bar) which may have a negative impact on the stability of Tretinoin.

The stability of the composition can be further enhanced by the addition of antioxidants such as tocopherol, butylated hydroxytoluene, butylated hydroxyanisole, ascorbyl palmitate, or edetates such as e.g. disodium edetate, with the edetates additionally binding possibly present heavy metals. The stability can furthermore be enhanced by the addition of preserving agents such as benzoic acid and parabens, e.g. methylparaben, and/or propylparabene. The desired pH is preferably stabilized by a buffer system. A citric acid buffer such as citric acid monohydrate or a phosphate buffer, particularly a buffer of potassium dihydrogen phosphate and disodium hydrogen are suitable.

The protons that are liberated upon thickening or cross-linkage, respectively, of the polyacrylate (e.g. Carbomer 974 P), are neutralized by the addition of a base, preferably sodium hydroxide.

One or more additional substances which have therapeutic affects on the skin may also be incorporated into the liposome compositions of the present invention. Such additional substances which may be incorporated include compounds capable of inducing epitheliazation, such as the chromanols such as Vitamin E. Anti-bacterial agents such as erythromycin, clindamycin, minocycline and the like may also be included for treatment of acne. Anti-inflammatory agents such as corticosteroids may also be advantageously included in the composition.

Very good penetration, particularly for Tretinoin as the effective substance, has been found for the following composition:

	g/100g
Tretinoin or analogous compounds	0.01-1.0
Lecithin hydrogenated (Soya)	1.0 - 10.000
Cholesterol	0.1 - 1.000
Ethanol	0.5 - 8.000
Butylated Hydroxytoluene	0.0 - 0.010
Methylparaben	0.0 - 0.150
Propylparaben	0.010 - 0.05
Citric Acid Monohydrate	0.0 - 0.5
Disodium edetate dihydrate	0.001 - 0.1
Sodium hydroxide	0.0 - 0.9
Carbomer 934 P	0.0 - 1.6
Water purified	ad 100.0

The liposome-based compositions of the present invention are prepared by applying the methods known in the art for manufacturing liposome compositions described in EP 253619, hereby incorporated by reference. In this method single bilayered liposomes are prepared by preparing an ethanolic solution of a phospholipid and the active ingredient and injecting the solution under pressure into an aqueous electrolyte solution contained in a high speed homogenizer. The liposomes are formed spontaneously providing liposomes having a diameter of less than 1μm. In particular, in accordance with the method of the present invention, the liposomes are manufactured by forming an aqueous electrolyte solution of the methylparaben, propylparaben and disodium edetate in purified water. Separately, the retinoic acid active ingredient, the lecithin and cholesterol are dissolved in an alcoholic solution such as ethanol. The aqueous solution is connected to a high performance homogenizer to effect circulation and the alcoholic solution containing the active ingredient is directly injected into the homogenizer. Liposomes of less than 1μm are formed spontaneously.

The particular advantages of the present invention are further illustrated by the following examples:

EXAMPLE 1

Liposome-Based Dispersion

A liposome-based dispersion of the following composition was produced according to the method described in EP 0 253 619:

Composition:

	g/100 g
Tretinoin	0.022
Lecithin (Soya) hydrogenated	5.000
Cholesterol	1.000
Ethanol	8.000
Tocopherol	0.010
Methylparaben	0.140
Propylparaben	0.010
Citric Acid Monohydrate	0.230
Sodium Hydroxide	0.440
Disodium edetate Dihydrate	0.100
Water purified	85.048

Procedure:

Methylparaben and propylparaben and the disodium edetate were dissolved in purified water at 80°C (kettle I). Tretinoin, tocopherol, lecithin, and cholesterol were dissolved in ethanol in a separate kettle (kettle II) at 55°C-70°C under agitation. The ethanol solution was purged with nitrogen during the whole procedure. The water phase was cooled to 55°C-70°C. Kettle 1 was connected to a high-performance homogenizer (Megatron MT-48; manufacturer: Kinematica, Littau, Lucerne, Switzerland) to effect circulation of the aqueous solution.

The ethanol solution was injected through a tube from kettle II directly into the homogenizer. Liposomes having a diameter of less than 1 mm were spontaneously formed and collected in kettle I.

Technical data:

Homogenizer speed: up to 13,000 rpm

Flow rate of the ethanol solution: 20-100 ml/s

EXAMPLE 2

Liposome-Based Gel

Composition:

	g/100 g
Tretinoin	0.022
Lecithin (Soya) hydrogenated	5.000
Cholesterol	1.000
Ethanol	8.000
Tocopherol	0.010
Methylparaben	0.140
Propylparaben	0.010
Citric Acid Monohydrate	0.230
Sodium Hydroxide	0.440
Disodium edetate Dihydrate	0.100
Carbomer 934 P	0.800
Water purified	84.248

Procedure:

The production of the liposome-based gel was performed as the one of the dispersion according to Example 1 with the exception that after the liposome formation according to Example 1 the Carbomer 934 P was admixed, followed by a sodium hydroxide solution.

Technical data:

Homogenizer speed: up to 13,000 rpm

Flow rate of the ethanol solution: 20 - 100 ml/s

EXAMPLE 3

Comparative tests on skin penetration

The skin penetration of Tretinoin from the products produced as described in Example was determined in vitro and compared with that of commercially available gel and cream products.

The penetration study was performed under the following conditions:

Diffusion Cells:	Franz Cell, 10 ml volume, 0.636 cm^2 surface area
Skin:	Male Hairless Mouse Skin (approximately 10 weeks old)
Receptor Media:	25% Isopropanol in pH 5.6 Buffer with 0.025% BHT
Conditions:	Cells covered with aluminum foil, under yellow light at 37°C
Study Duration:	24-26 hours

Amount of Formulation Applied: Approximately 1 ml

Skin samples were mounted onto Franz Diffusion Cells, and approximately 1 ml of each formulation was applied and spread evenly on the skin surface. At the end of the study period the receptor solution was sampled (0.15ml), and excess formulation was wiped from the skin surface using a "Kim-Wipe" (the Kim-Wipe was then extracted to recover the retinoid).

Skin samples were extracted by placing them in a 50ml Volumetric Flask and filled to 50 ml with methanol:ethyl acetate (1:1). The flasks were then sonicated for 2 hours and the solutions assayed for retinoid. Following extraction, the ethanol solution was assayed for retinoid by HPLC with UV detection at 325nm.

Results and Discussion

A summary of the amount of tretinoin remaining in the skin after 24 hours for each formulation investigated is shown in FIG. 1.

The results from the in vitro skin penetration study indicate that the gel formulation delivered the most tretinoin to the skin (22.6%) when compared to the other formulations. The application of the liposomal gel of Example 2 above resulted in the second highest tretinoin skin levels (6%) when compared to the other formulations. The cream formulation (a W/O emulsion) had the lowest levels (3%).

A summary of the amount of drug permeating through the full thickness of the skin and entering into the receptor media is shown in FIG. 2.

The results indicate that a large amount of the gel dose (18%) was found in the receptor after 24 hours. In contrast, the liposomal and cream formulations produced no detectable levels of tretinoin in the receptor, suggesting that a vast majority of the applied dose remained in or on the skin. Most significantly, the liposomal gel of the present invention achieved tretinoin skin levels approximately 4-fold higher than the cream formulation yet no tretinoin was detected in the receptor compartment. These results suggest that the liposomes may be preferentially delivering the tretinoin to the skin but not through the skin.

EXAMPLE 4

Stability Testing

Four batches of liposomal tretinoin formulation were manufactured in accordance with the procedure of Example 1. Batches 12, 13 and 14 were manufactured with purified soyabean lecithin and hydroxypropyl methylcellulose as gelling agent. Batch 16 was manufactured with hydrogenated soyabean lecithin and

Carbomer as the gelling agent. The strengths and compositions of the batches are shown in Table 1.

TABLE 1

Formulation	Composition	Strength (%Tretinoin)
12	purified lecithin/HPMC	0.01%
13	purified lecithin/HPMC	0.025%
14	purified lecithin/HPMC	0.05%
16	Hydrogenated lecithin/HPMC	0.02%

The batches were assayed for stability at various time intervals for total tretinoin content and liposomally bound tretinoin content by liquid chromatographic method. The results are set forth in Tables 2-5.

FORMULATION 12 TABLE 2

	Total content of tretinoin (in % of declaration)			Liposomally bound content of tretinion (in % of declaration)		
Storage (months)	4°C	25°C	30°C	4°C	25°C	30°C
Initial	107.7	107.7	107.7	99.8	99.8	99.8
6		109.3	111.4/106.5*		92.9	83.7/88.1*
11		104.8	103.1			
13		107.1	95.8		86.9/89.0*	77.8
18		124.8	95.2/89.6*		105.5	85.5

FORMULATION 13 TABLE 3

	Total content of tretinoin (in % of declaration)			Liposomally bound content of tretinion (in % of declaration)		
Storage (months)	4°C	25°C	30°C	4°C	25°C	30°C
Initial	108.7	108.7	108.7	102.8	102.8	102.8
6		108.5	107.5		93.7	89.2/98.7*
11		106.2	98.6/101.3*			
13		110.3	99.6		88.3/92.4*	84.4/81.7*
18		121.4	99.8		105.0	83.5/89.6*

FORMULATION 14 TABLE 4

	Total content of tretinoin (in % of declaration)			Liposomally bound content of tretinion (in % of declaration)		
Storage (months)	4°C	25°C	30°C	4°C	25°C	30°C
Initial	105.4	105.4	105.4	89.2	89.2	89.2
10		106.8	99.0			
12		108.5	103.4		108.4	103.9
20		102.2	93.3		108.3	98.2/91.2*

5

FORMULATION 16 TABLE 5

	Total content of tretinoin (in % of declaration)			Liposomally bound content of tretinion (in % of declaration)		
Storage (months)	4°C	25°C	30°C	4°C	25°C	30°C
Initial	109.6	109.6	109.6	109.6	109.6	109.6
1		109.3	108.7		107.4	108.8
3	105.7	106.5	106.2	104.2	107.8	105.2
20	105.7	106.5	103.7	104.2	103.5	100.4
24	106.4	104.9		98.9	98.8	
36**	107.3	107.5		109.7	107.8	
50**	110.6	107.3		110.1	106.9	
61**	115.0	110.2		113.9	112.4	

Conclusion

The data demonstrates a good stability of up to five years for formulation 16 when stored in aluminum tubes at 25°C. Stability investigations for formulations 12, 13 and 14 were discontinued after 20 months storage time due to sample inhomogeneities.

Claims

We claim:

1. A liposome-based composition for use in the topical treatment of skin disorders comprising:
   (a) an effective amount of an active ingredient comprising tretinoin or a derivative thereof;
   (b) a lipidic phase comprising:
      (i) lecithin or hydrogenated lecithin; and
      (ii) cholesterol or a derivative thereof selected from cholesterol esters, polyethylene glycol derivatives of cholesterol (PEG-cholesterols), and organic acid derivatives of cholesterols; and
   (c) a lower alcohol (preferably ethanol);

   wherein the composition comprises single bilayered liposomes made by preparing a solution of the lipidic phase and the active ingredient in the alcohol and injecting the solution under pressure into an aqueous electrolyte solution contained in a high speed homogenizer.

2. The liposome-based formulation of claim 1, wherein the lower alcohol is ethanol.

3. The liposome-based formulation of claim 1, characterized in that it comprises furthermore at least one polyacrylate.

4. The liposome-based formulation of claim 3, wherein the polyacrylate is carbomer.

5. The liposome-based formulation of claim 1, wherein the lecithin is hydrogenated lecithin.

6. The liposome-based formulation of claim 1, characterized in that it furthermore comprises a preserving agent

7. The liposome-based formulation of claim 1, characterized in that it furthermore comprises an antioxidant.

8. The liposome-based formulation of claim 1, characterized in that it furthermore comprises a complexing agent.

9. The liposome-based formulation of claim 2, characterized in that it has the following composition:

	g/100g
Tretinoin or its derivatives	0.01-1.0
Lecithin hydrogenated (Soya)	1.0 - 10.000
Cholesterol	0.1 - 1.000
Ethanol	0.5 - 8.000
Butylated Hydroxytoluene	0.0 - 0.010
Methylparaben	0.0 - 0.150
Propylparaben	0.010 - 0.05
Citric Acid Monohydrate	0.0 - 0.5
Disodium edetate dihydrate	0.001 - 0.1
Sodium hydroxide	0.0 - 0.9
Carbomer 934 P	0.0 - 1.6
Water purified	ad 100.0

10. The liposome-based formulation of claim 1 for use as a pharmaceutical preparation for the treatment of acne.

11. The liposome-based formulation of claim 1 for use as a pharmaceutical preparation for the treatment of photoaging or wrinkles.

12. The liposome-based formulation of claim 2, characterized in that it has the following composition:

	g/100 g
Tretinoin	0.022
Lecithin (Soya) hydrogenated	5.000
Cholesterol	1.000
Ethanol	8.000
Tocopherol	0.010
Methylparaben	0.140
Propylparaben	0.010
Citric Acid Monohydrate	0.230
Sodium Hydroxide	0.440
Disodium edetate Dihydrate	0.100
Carbomer 934 P	0.800
Water purified	84.248

Figure 1

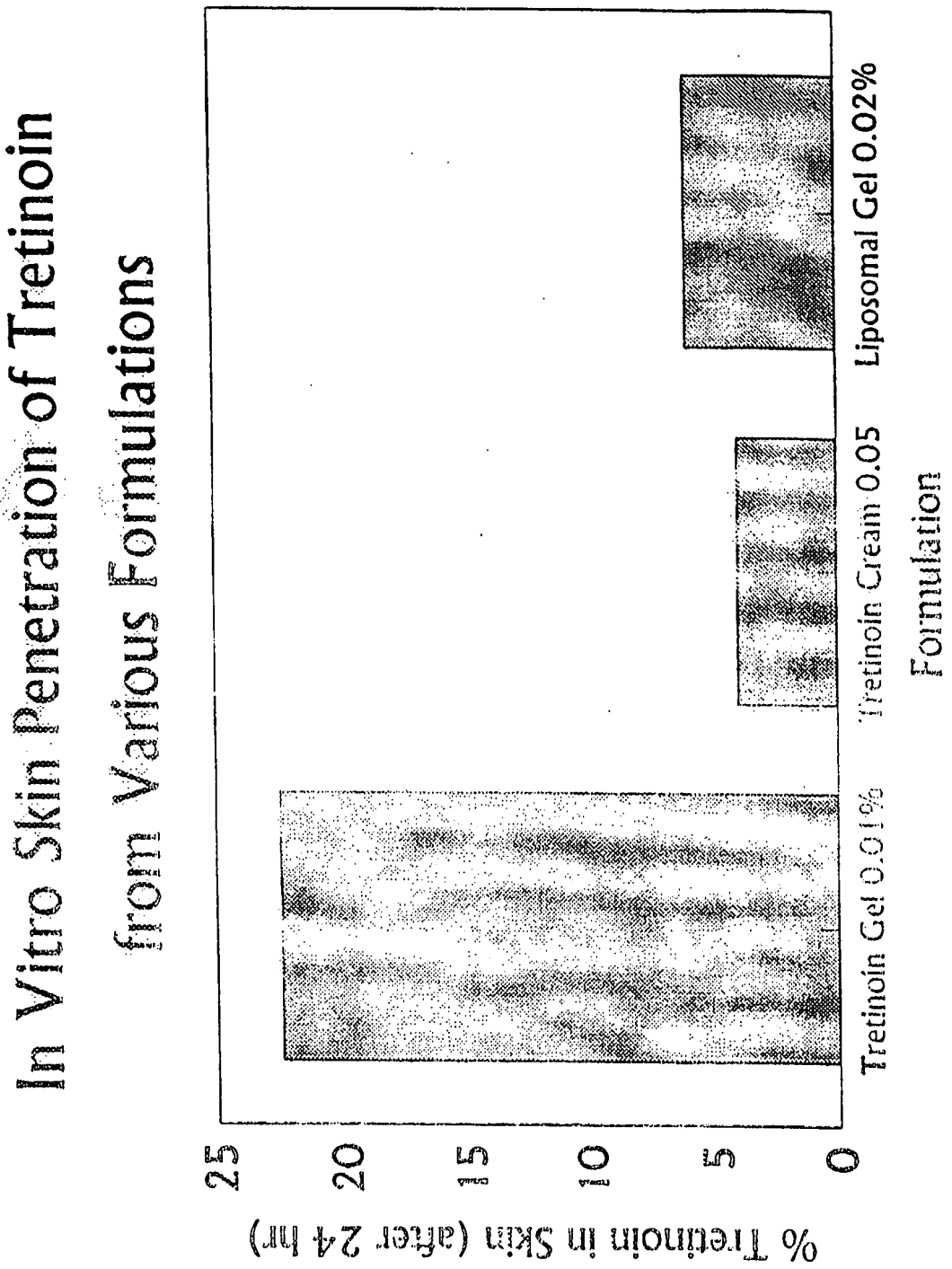
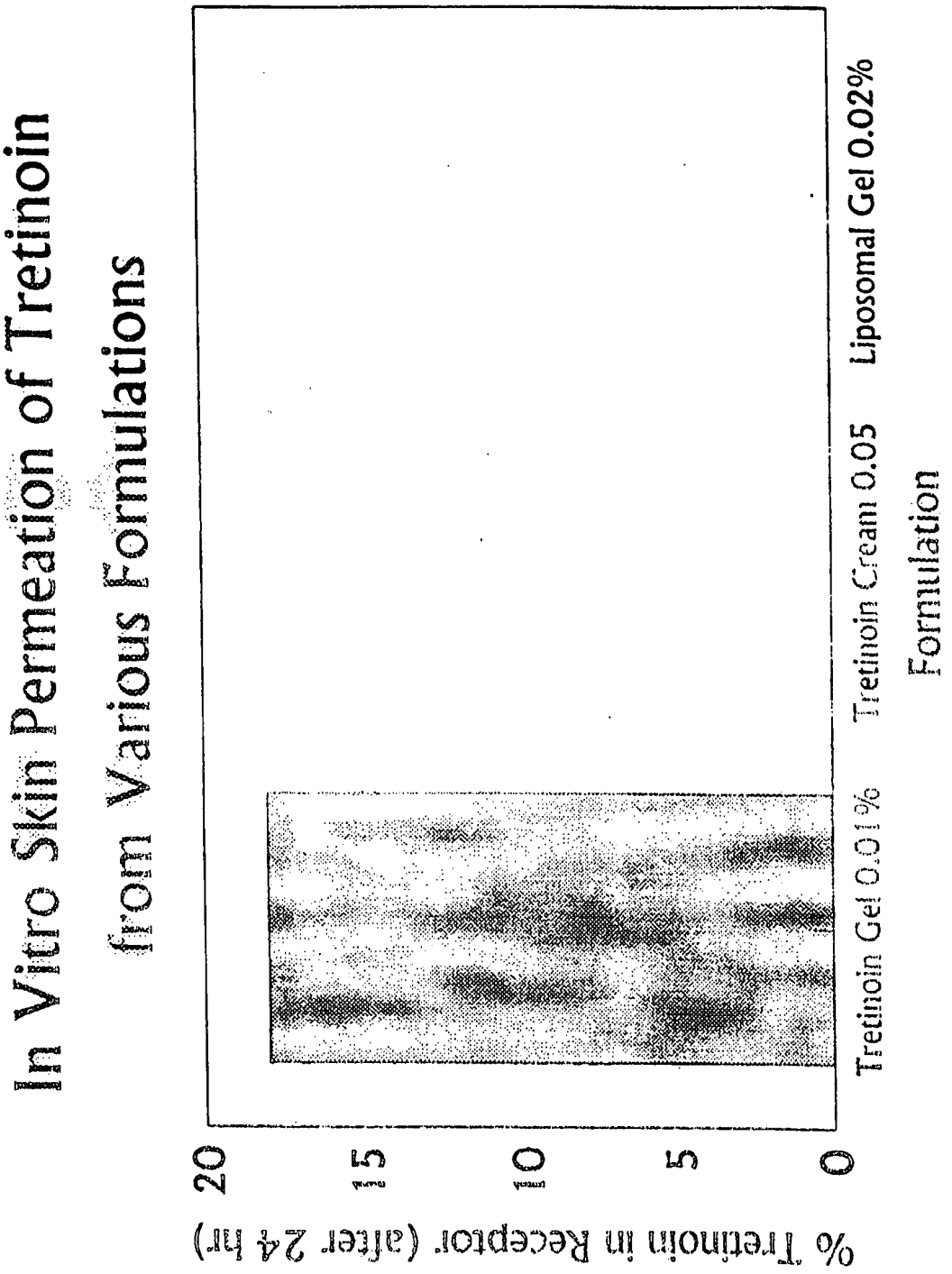

In Vitro Skin Penetration of Tretinoin
from Various Formulations
% Tretinoin in Skin (after 24 hr)
0
5
10
15
20
25
Tretinoin Gel 0.01%
Tretinoin Cream 0.05
Liposomal Gel 0.02%
Formulation

Figure 2


In Vitro Skin Permeation of Tretinoin
from Various Formulations
% Tretinoin in Receptor (after 24 hr)
20
15
10
5
0
Tretinoin Gel 0.01%
Tretinoin Cream 0.05
Liposomal Gel 0.02%
Formulation

INTERNATIONAL SEARCH REPORT

International Application No PCT/IB 98/00206

A. CLASSIFICATION OF SUBJECT MATTER

A 61 K 31/20,A 61 K 9/133,A 61 K 9/06

According to International Patent Classification (IPC) or to both national classification and IPC6

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A 61 K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0253619 A2 (CILAG LTD) 20 January 1988 (20.01.88), claims 1-3,11-14, column 3, lines 45-53, column 4, lines 31-56, column 5, lines 8-15, column 5, line 54 - column 6, line 2 (cited in the application).	1,2,6, 7,10, 11
Y	Claims 1-3,11-14, column 3, lines 45-53, column 4, lines 31-56, column 5, lines 8-15, column 5, line 54 - column 6, line 2.	3-5,8, 9,12
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Y	US 5034228 A (MEYBECK, A. et al.) 23 July 1991 (23.01.91), claims 1,3,12,16-22,	3-5,8, 9,12

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 May 1998	12.06.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	MAZZUCCO e.h.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No PCT/IB 98/00206

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	abstract, column 2, line 53 - column 3, line 22, examples 3-6,13,14 (cited in the application). --	
X	WO 95/35095 A1 (YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM) 28 December 1995 (28.12.95), abstract, claims 1-3,5,6, page 3, lines 10-17, page 7, lines 2-6. --	1-5, 10,11
A	WO 90/14833 A1 (BAZZANO, G.) 13 December 1990 (13.12.90), claims 1-16. --	1-12
A	MAINGNEN, F. et al. Mise au point, developpement d'une formulation de liposomes de tretinoine pour la realisation d'un essai clinique dans le sarcome de Kaposi. Journal De Pharmacie Clinique, June 1995, Vol. 14, No. 2, pages 137-138, the whole article (cited in the application). ----	1

ANHANG

zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

ANNEX

to the International Search Report to the International Patent Application No.

ANNEXE

au rapport de recherche international relatif à la demande de brevet international n°

PCT/IB 98/00206 SAE 184610

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The Office is in no way liable for these particulars which are given merely for the purpose of information.

La présénte annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche international visée ci-dessus. Les renseignements fournis sont donnés à titre indicatif et n'engagent pas la responsibilité de l'Office.

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